

In re Application of:  
Short and Keller  
Application No.: 08/876,276  
Filed: June 16, 1997  
Page 3

PATENT  
Attorney Docket No.: DIVER1280

**Amendments to the Claims:**

Please amend claims 19 and 40 as follows.

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims**

Claims 1-18. (Cancelled)

19. (Currently Amended) A method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

- a) normalizing the representation of organisms present in a sample containing naturally occurring DNA from more than one organism to ~~increase representation of rare species~~ allow equal representation of the DNA from all of the organisms present in the sample;
- b) contacting a bioactive substrate that is fluorescent in the presence of the bioactivity or biomolecule of interest with a library containing a plurality of clones containing the normalized DNA prepared in a) under conditions suitable for at least one clone to express a bioactivity or biomolecule of interest, wherein each clone contains DNA from a single organism;
- c) screening the library with a fluorescent analyzer that detects bioactive fluorescence; and
- d) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of a naturally occurring DNA that encodes a bioactivity or biomolecule of interest.

20. (Previously Presented) The method of claim 19, further comprising obtaining DNA from a clone that is positive for an enzymatic activity of interest.

GTV6439203.1  
104703-156676

In re Application of:  
Short and Keller  
Application No.: 08/876,276  
Filed: June 16, 1997  
Page 4

PATENT  
Attorney Docket No.: DIVER1280

21. (Previously Presented) The method of claim 20, wherein the enzymatic activity of interest is from an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.
22. (Previously Presented) The method of claim 19, wherein the library is generated in a prokaryotic cell.
23. (Previously Presented) The method of claim 19, wherein the library contains at least about  $2 \times 10^6$  clones.
24. (Previously Presented) The method of claim 22, wherein the prokaryotic cell is gram negative.
25. (Previously Presented) The method of claim 19, wherein the clones are encapsulated in a gel microdrop.
26. (Previously Presented) The method of claim 19, wherein the analyzer screens up to about 15 million clones per hour.
27. (Previously Presented) The method of claim 19, wherein the clones are extremophiles.
28. (Previously Presented) The method of claim 27, wherein the extremophiles are thermophiles.
29. (Previously Presented) The method of claim 27, wherein the extremophiles are hyperthermophiles, psychrophiles, halophiles, psychrotrops, alkalophiles, or acidophiles.
30. (Previously Presented) The method of claim 19, wherein the bioactive substrate comprises staining reagent C12-fluorescein-di-D-galactopyranoside (C12FDG).

GT6439203.1  
104703-156676

In re Application of:  
Short and Keller  
Application No.: 08/876,276  
Filed: June 16, 1997  
Page 5

PATENT  
Attorney Docket No.: DIVER1280

31. (Previously Presented) The method of claim 19, wherein the bioactive substrate comprises a lipophilic tail.
32. (Previously Presented) The method of claim 19, wherein the clones and substrates are heated to enhance contacting of the substrate with the clones.
33. (Previously Presented) The method of claim 32, wherein the heating is to a temperature of about 70°C.
34. (Previously Presented) The method of claim 32, wherein the heating is for about 30 minutes.
35. (Previously Presented) The method of claim 19, wherein the fluorescent analyzer comprises a fluorescence activated cell sorting (FACS) apparatus.
36. (Previously Presented) The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA is stable at a temperature of at least about 60°C.
37. (Previously Presented) The method of claim 19, wherein the library is an expression library.
38. (Previously Presented) The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA possesses enhanced enzymatic activity of interest compared to that of a wild-type enzyme.
39. (Previously Presented) The method of claim 19, wherein the method further comprises biopanning the expression library prior to contacting with the substrate.

GTV6439203.1  
104703-156676

In re Application of:  
Short and Keller  
Application No.: 08/876,276  
Filed: June 16, 1997  
Page 6

PATENT  
Attorney Docket No.: DIVER1280

40. (Currently Amended) The method of claim 19 further comprising obtaining DNA from a clone identified in step [[c]]d that is positive for an enzymatic activity of interest and comparing the enzymatic activity of a DNA expression product from the clone with that obtained from such a clone into whose DNA at least one nucleotide mutation has been introduced, wherein a difference in enzymatic activity is indicative of the effect upon the enzymatic activity of interest caused by introduction of the at least one nucleotide mutation.

41. (Previously Presented) The method of claim 40, wherein the enzyme encoded by the DNA possesses the enzymatic activity of interest at a temperature at least 10 °C below the temperature of optimal activity of the bioactivity encoded by a corresponding wild-type DNA.

Claim 42. (Cancelled)

43. (Previously Presented) The method of claim 19, wherein the library is a multispecies library.

44. (Previously Presented) The method of claim 43, wherein the library is generated from a mixed population of uncultured organisms.

45. (Previously Presented) The method of claim 43, wherein the library is generated from isolates.

46. (Previously Presented) The method of claim 40, wherein the mutation is introduced by error-prone PCR, oligonucleotide directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis and exponential ensemble mutagenesis.

GT6439203.1  
104703-156676